Br. J. clin. Pharmac. (1993), **35**, 136–142

The bioavailability of digoxin from three oral formulations measured by a specific h.p.l.c. assay

A. F. COHEN[1], R. KROON[1], H. C. SCHOEMAKER[1], D. D. BREIMER[2], A. VAN VLIET-VERBEEK[1] & H. C. BRANDENBURG[3]

[1]Centre for Human Drug Research, Leiden University Hospital, [2]Center for Bio-Pharmaceutical Sciences, Leiden University and [3]Department of Clinical Pharmacy, Leiden University Hospital, Leiden, The Netherlands

1 We have studied the absolute bioavailability of three oral formulations of digoxin, 1.0 mg, in 12 young healthy volunteers in a four way randomised cross-over study using an intravenous control.

2 Digoxin tablets (250 μg), liquid filled digoxin capsules (100 μg) and an experimental enteric-coated capsule (100 μg) were evaluated. *In vitro* dissolution at pH 1 demonstrated extensive hydrolytic breakdown of digoxin from the tablets and capsules but not from the enteric-coated capsules.

3 Serum 'digoxin' concentrations were measured by fluorescence polarization immunoassay (FPI). The systemic availability (± s.d.) of the capsules was 70.5 ± 11.3%, and that of the tablets 71.5 ± 8.6%. Drug was less available from the enteric-coated capsules (62.1 ± 10.3%) measured with FPI. These results were reflected in the urinary drug recoveries measured by FPI.

4 By contrast, there were no differences in urinary recovery of unchanged digoxin between any of the oral treatments, when this was measured by h.p.l.c. The cross-reactivity of immunoassays for metabolites of digoxin may produce artefactual results and the optimal pharmaceutical formulation for digoxin remains to be determined.

Keywords digoxin bioavailability metabolism

Introduction

The bioavailability of digoxin can be variable and depends on the pharmaceutical formulation (Johnson *et al.*, 1976) and the method of its preparation. This has led to differences in drug availability from tablets (Lindenbaum *et al.*, 1971). The finding that the *in vitro* rate of dissolution of tablets could be used to predict the amount absorbed *in vivo* has led to quality control criteria for solid dosage forms of digoxin. Liquid-filled capsules are claimed to have almost complete availability (Doherty *et al.*, 1984). The bioavailability of digoxin has generally been assessed with radioimmunoassay. However, there is evidence that digoxin is metabolised to a large extent (Gault *et al.*, 1982; Magnusson *et al.*, 1982) and that it is degraded by acid hydrolysis in the stomach (Cohen *et al.*, 1991; Gault *et al.*, 1980, 1981; Sonobe *et al.*, 1980) to products with a shorter half-life (Kuhlmann *et al.*, 1987; Loo *et al.*, 1977) than digoxin and in the case of digoxigenin considerably reduced cardiac activity (Bottcher *et al.*, 1973; Lullmann & Peters, 1971; Marcus *et al.*, 1975). These breakdown products are determined by all immunological assays as unchanged digoxin and may confound the results of bioavailability studies. In this study we measured the systemic availability and the effects of conventional tablets, liquid-filled capsules and a new enteric-coated, liquid-filled capsule, in comparison with an intravenous infusion.

We hypothesized that the hydrolysis of digoxin released from conventional tablets and liquid-filled capsules may be more extensive than from the enteric-coated capsules. This would lead to comparable plasma and urine concentrations, when measured by immunoassay, thereby concealing potential differences in unchanged digoxin as measured using a selective chromatographic assay.

Correspondence: Dr A. F. Cohen, Centre for Human Drug Research, University Hospital, P.O. Box 9600, 2300 RC Leiden, The Netherlands

Methods

Subjects

We recruited 12 subjects (6M, 6F; age 20–39 years; weight 55–88 kg). They were healthy as ascertained by history, physical examination, routine biochemistry, haematology, urinalysis and an ECG. They gave written consent to a protocol which was approved by the Medical Ethics Committee of Leiden University Hospital.

Study design

The study was an open, randomised, cross-over trial of four dosage forms, each containing 1 mg digoxin, given at 2 weekly intervals. The order of treatment administration was determined by three balanced 4 × 4 Latin squares. The dosage forms were:

A. Digoxin tablets 4 × 0.25 mg (Lanoxin®—Wellcome).
B. Digoxin capsules 10 × 0.1 mg (Lanoxicaps®—Wellcome).
C. Digoxin capsules 10 × 0.1 mg with enteric coating.
D. Intravenous digoxin 1.0 mg (Lanoxin®) administered at a constant rate over 1 h.

All of the oral treatments were given with 50 ml tap water. The intravenous infusion was given as a 50 ml solution in saline with an infusion pump (Harvard Pump 22—Harvard Instruments, Edenbridge, England). The enteric-coated capsules were prepared in the pharmacy of Leiden University Hospital by applying a coating of Eudragit-L to liquid-filled capsules using standard methods.

Protocol

The subjects came to the Research Unit in the morning after an overnight fast from 22.00 h. They were allowed to drink water freely up to 1 h before drug administration, but were not allowed caffeine-containing drinks during the study day and were not allowed to smoke. They took a light standard lunch at 12.30 h. Alcohol was not allowed from 24 h before the study to the end of the study day. During the urine sampling period after the first study day alcohol was allowed in moderate amounts. Intravenous cannulae were inserted in forearm veins for blood sampling and (if appropriate) infusion and kept patent with heparinised saline. Blood samples after 12 h were taken by venepuncture. Blood samples for digoxin were taken before administration and at 0.25, 0.50, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 h afterwards. Urine was collected in 2 h collection periods until 6 h, then from 6–10 h, and from 10–12 h at the Research Unit, and then at home from 12–24 h and over 24 h periods until 96 h. Cardiovascular effects were measured at 1, 2, 3, 4, 6, 8 and 10 h after drug administration.

Blood samples were taken in 5 ml plain tubes (Sarstedt monovette®) and left to clot. Serum was separated and stored at −40° C until analysis.

All urine passed was collected in plastic containers (Sarstedt) and handed in at the Research Unit as soon as possible. Volumes were determined by weight and samples for digoxin assay were kept in plastic tubes. An additional aliquot was taken for creatinine determination as a check on the completeness of the urine collection.

Creatinine was measured by a standard method in the Department of Clinical Chemistry, Leiden University Hospital.

Digoxin assays

Digoxin in urine was measured by h.p.l.c. with fluorometric detection (Shepard *et al.*, 1986). This method involves the extraction of digoxin and its metabolites with methylene chloride followed by derivatization with 1-naphthoyl chloride with 4-dimethylammonium chloride as a catalyst. Digitoxin was used as the internal standard. The method has a detection limit (defined at three times the noise level in the chromatogram) of digoxin of 10 ng ml^{-1} (0.13 nmol l^{-1}) and a coefficient of variation of 3.9% at a concentration of 124 nmol l^{-1}. This method is not sufficiently sensitive for the analysis of digoxin in serum. Digoxin (D3), its bis-digitoxoside(D2), mono-digitoxoside(D1) and digoxigenin(D0) could be separated and detected by this procedure. In addition, the reduced metabolite dihydrodigoxin could also be detected. All recoveries are expressed in nmol. (The dose of 1 mg digoxin corresponds to 1280 nmol of digoxin).

Urine and serum samples were also analysed by fluorescence polarization enzyme immunoassay (FPI) (TDx Digoxin II) with an automatic analyser (TDx, Abbott Laboratories, Diagnostics Division, North Chicago, Il, USA) using the standard method for plasma or after appropriate dilution of the urine samples. This immunoassay uses an antibody that has 205% cross-reactivity for D0, 150% for D1, and 115% for D2 (data from the manual for the TDx assay). Urinary recoveries have been expressed in similar units as for the h.p.l.c assay.

For each sample the ratio of the values measured by FPI and h.p.l.c. was calculated.

Dissolution tests of dosage forms

Dissolution tests were performed according to the standard method described in the US Pharmacopoea (ed XXI). The test uses a rotating wire basket in a vessel with 1000 ml of dissolution medium. The capsules and tablets were tested in medium at pH 1. The enteric-coated capsule was added to the dissolution medium at pH 1 for 2 h and the pH was subsequently raised to 6.8. Samples were taken from the dissolution medium at 2–5 min intervals and immediately neutralised with borate buffer pH 10 to prevent further hydrolysis. The USP method specifies analysis with a non-specific fluorometric assay but for this study samples were analysed by the same h.p.l.c. method as used for urine. All formulations were tested in triplicate.

Effect measurements

Blood pressure and heart rate were measured with an automatic oscillometric system (Nihon Kohden

MPCV-7201). Systolic time intervals were measured from a simultaneous recording of a single ECG lead, phonocardiogram, and carotid pulse curve using an eight channel polygraph (Nihon-Kohden) with appropriate amplifiers and transducers. Signals were recorded with an eight-channel thermal array recorder (Nihon-Kohden) with a frequency response of 2.8 kHz and by a computer system (Cambridge Electronics Design, LCVM system with CED1401 interface) which allowed on-screen measurement of time intervals. Systolic time intervals (QS_2, LVET and PEP) were measured from at least 5 beats by standard methods and subsequently corrected for heart rate where appropriate (QS_2I, LVETI, and PEP/LVET ratio) (Warrington *et al.*, 1989).

Electrocardiographic measurements were made from twelve lead ECGs with a Nihon Kohden Cardiofax V ECG 8240A cardiograph with ECAPS 12 analysis software (Nihon-Kohden, Amsterdam, The Netherlands). The lead with the highest T wave amplitude was selected at screening and all measurements were made from this lead (V3 in seven subjects, V4 in three, and II and V2 in the remaining two). PR, QRS, and QT durations were measured. In addition, the amplitude of the T wave and the ST segment at the J point, at the midpoint, and at the end were evaluated.

Pharmacokinetic and statistical analyses

Terminal half-lives of digoxin after i.v. administration were determined by nonlinear regression analysis using a bi-exponential model. In addition, the plasma half-life of digoxin was estimated from a plot of the rate of urinary excretion of digoxin *vs* time. Calculations were performed using the software package 'Siphar' (Simed, Creteil, France). The area under the serum drug concentration-time plot (AUC) was calculated by the linear trapezoidal rule without extrapolation to infinity. Systemic availability (F) based upon serum drug concentration was calculated as the ratio of the AUC for the dosage form to the AUC after i.v. administration. Systemic availability from urine data was calculated as the ratio of the cumulative amounts of the oral and i.v. doses that were excreted over 96 h.

Effect measurements were analysed as the area under the effect-time curve, which was calculated by the trapezoidal rule and subsequently divided by the time interval. For effects and the plasma AUC separate analyses were made for the period from pretreatment to 120 min and for the full study period.

Statistical analysis was performed by repeated measures analysis of variance followed by orthogonal comparisons (paired t-tests), comparing the average of the oral treatments with the intravenous treatment, the average of the uncoated oral treatments with the enteric-coated capsule, and the uncoated capsule with the uncoated tablet, when significant treatment effects were detected on the overall analysis of variance. Values are reported with 95% confidence intervals of the difference. Calculations were carried out using SPSS/PC + V3.0 statistical software (SPSS Inc, Chicago, Il).

Results

All of the subjects completed the study. Subject 2 vomited within 1 h of taking the digoxin tablets; the study was discontinued and repeated 2 weeks later without changing the treatment order. Subjects 1 and 10 felt sick and vomited at 2 h or more after tablet administration, but it was decided to include the data from these subjects, since the examination of the cumulative excretion of digoxin showed that absorption or transit from the stomach to the intestine had already taken place.

Completeness of urine collection was monitored by creatinine excretion. Some subjects reported loss of urine (one subject dropped a bottle and one subject accidentally overturned one) and in these cases volume was corrected using the creatinine excretion measured on the other occasions. Subjects 2 and 9 accidentally received 0.5 instead of 1.0 mg of i.v. digoxin. Their data were not included in the statistical analysis.

Dissolution tests

Both the tablet and the capsule were extensively degraded in acid dissolution medium (Figure 1a and 1b). These formulations had released their contents within 10 min and degradation of 50% of the original content to digoxigenin occurred within 20 min. The enteric-coated capsule remained unopened for 120 min at pH 1 and released its contents within 30 min after the pH of the medium had been made neutral (Figure 1c). Digoxin content remained constant afterwards, demonstrating the absence of hydrolysis at this pH.

Serum concentrations of digoxin measured with FPI

The average serum concentrations of digoxin are shown in Figure 2 and the average data in Table 1. The AUC(0–72 h) was significantly lower (3057 nmol l^{-1} h) after the oral treatments than after i.v. administration (4499 nmol l^{-1} h; 95% CI of the difference 1150–1730 nmol l^{-1} h) and the AUC of the enteric-coated capsule (2894 nmol l^{-1} h) was 480 nmol l^{-1} h (120–830) lower than that after the other oral (uncoated) formulations. Differences in systemic availability among the oral formulations did not reach statistical significance (Table 1). In the first 2 h after administration much greater differences were seen. The i.v. treatment produced a greater AUC than the oral treatments. The AUC after the enteric-coated capsules was 46.8 nmol l^{-1} h, which was lower than the average of the other oral treatments by 445 nmol l^{-1} h (330 − 560). In addition, the tablet gave a smaller AUC than the uncoated capsules; 402 *vs* 582 nmol l^{-1} h (95% CI 40–320).

Urinary recovery of digoxin measured by h.p.l.c.

Using the specific h.p.l.c. method 68.4 ± 15.4% (range 48.4–102%) of the total dose by the intravenous route was recovered in the urine in 96 h. The average recovery after i.v. administration was 875.8 nmol, which was significantly higher by 352.9 nmol (95% CI 232.2–473.6) than after the oral treatments. In contrast

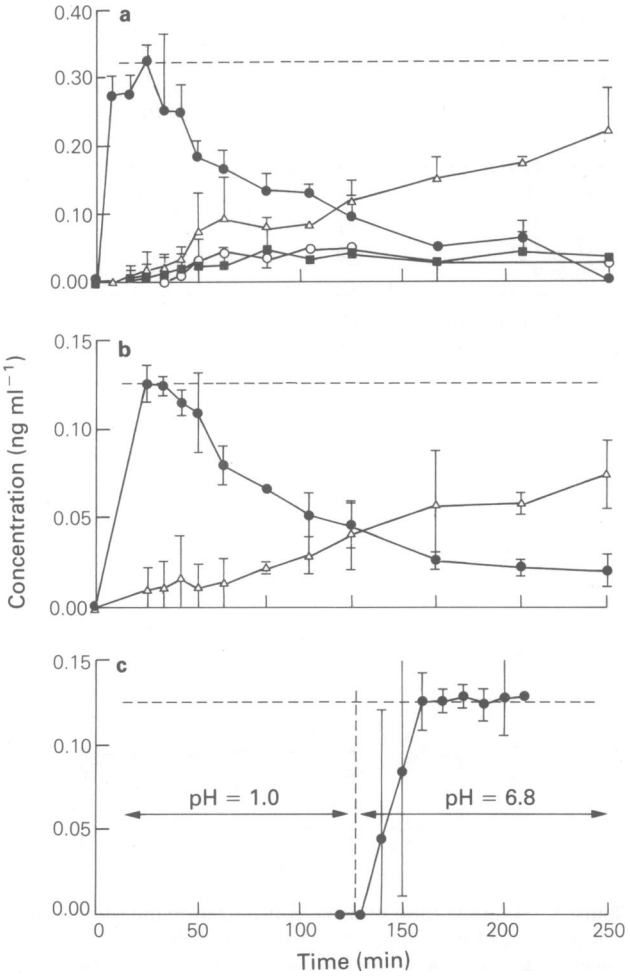

Figure 1 Results of dissolution tests on the dosage forms using the rotating basket method (USP ed XXI). Data for the tablet are shown in the top panel, for the capsule in the middle panel and for the enteric-coated capsule in the bottom panel. Average (± s.d.) values for triplicate determinations are shown. The tablet and the capsule were tested at pH 1 and the enteric-coated capsule was subjected to a pH change after 2 h. ● = digoxin; △ = digoxigenin; ■ = mono-digitoxoside; ○ = bis-digitoxoside. An artefact prevented measurement of two breakdown products from the capsule.

to the results in plasma there were no significant differences in the recovery on systemic availability of unchanged digoxin among the oral treatments (Table 2).

Plots of cumulative drug excretion *vs* time demonstrated that the difference between i.v. and the other treatments originated from the beginning of the experiment (Figure 3a).

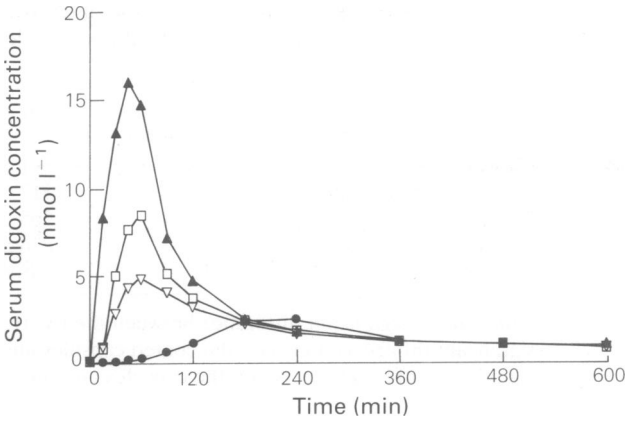

Figure 2 Average serum concentrations of digoxin measured by FPI immunoassay. ▲ = 1 mg i.v.; □ = liquid-filled capsules; ▽ = tablets; ● = enteric-coated capsule. Data are shown up to 10 h for clarity, but were collected until 72 h.

Urinary recovery of digoxin measured by FPI

Urinary data paralleled the serum data with respect to estimation of bioavailability (Table 2). The recovery was 716.6 ± 114.1 nmol after i.v. treatment, significantly higher than after the oral treatments. The recovery from the enteric-coated capsule was 447.7 ± 148.8 nmol, significantly lower than that from the uncoated oral treatments; 77.6 nmol (19.3–136.0). There was no difference in recovery between the capsules and the tablets. This is shown in Figure 3b, again demonstrating that differences developed at the start of treatment.

Urinary half-life

The urinary half-life was determined from the excretion rate-time plot measured by FPI. The average (± s.d.) half-life after the tablets was 47 ± 23 h, after the uncoated capsules 38 ± 20 h and after the enteric-coated capsules 47 ± 28 h. The half-life in plasma after the i.v. injection was 42 ± 9 h. There were no significant differences among any of these treatments.

Ratio of h.p.l.c. and FPI urine measurements

In the first hours after the administration of tablets and liquid-filled capsules the ratio of h.p.l.c. to FPI urine measurements exceeded one and returned to unity at later times (Figure 4). The ratio showed considerable

Table 1 Mean (± s.d.) areas under the serum drug concentration curve (AUC) from 0–72 h and from 0–120 min for the different treatments, measured by FPI

	AUC (nmol l⁻¹ h)		*Systemic availability (%)*
	0–120 min	*0–72 h*	*0–72 h*
Capsules	582 ± 292	3447 ± 1035	70.5 ± 11.3
Enteric-coated capsules	47 ± 25**	2894 ± 633**	62.1 ± 10.3
Tablets	402 ± 121†	3295 ± 649	71.5 ± 8.6
Intravenous injections	1291 ± 364*	4499 ± 893*	—

* = difference between i.v. and all oral treatments ($P < 0.001$); ** = difference between enteric-coated capsule and the oral uncoated treatments ($P = 0.013$); † = difference between uncoated capsules and tablets ($P = 0.019$). There was no significant difference between the capsule and the tablet from 0–72 h.

Table 2 The cumulative urinary excretion of digoxin over 96 h measured by h.p.l.c. and immunoassay (FPI) after the different treatments. The bioavailability of the dosage forms is calculated in comparison with i.v. treatment

	Cumulative excretion (nmol 96 h^{-1})		*Systemic availability (%)*	
	H.p.l.c.	*FPI*	*H.p.l.c.*	*FPI*
Capsules	541 ± 182	532 ± 150	62 ± 22	69 ± 16
Enteric-coated capsules	517 ± 172	448 ± 149**	59 ± 16	58 ± 17
Tablets	565 ± 160	518 ± 125	62 ± 14	67 ± 11
Intravenous injection	876 ± 197*	717 ± 114*		
Percentage of i.v. dose recovered	68 ± 15	56 ± 9		

* indicates a significant difference between the i.v. treatment and all oral treatments ($P < 0.001$). ** signifies a significant difference between the coated capsules and the uncoated oral treatments ($P = 0.014$). There were no significant differences between the capsules and the tablets.

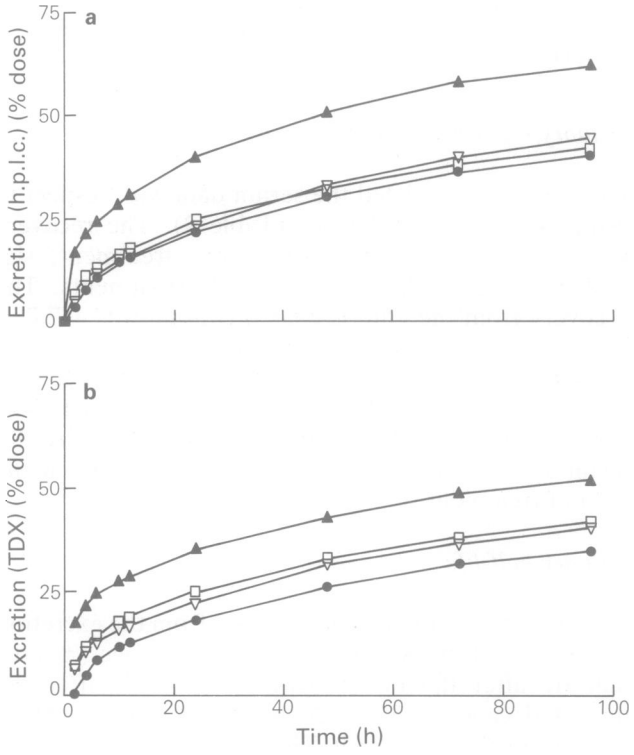

Figure 3 Cumulative urinary excretion of digoxin as percentage of the total dose measured by h.p.l.c. (a) and FPI (b). ▲ = 1 mg i.v.; □ = liquid-filled capsules; ▽ = tablets; ● = enteric-coated capsule. I.v. treatment gave the highest recovery after both methods but the enteric-coated capsule had a significantly reduced systemic availability compared with the other oral treatments by FPI, but not by h.p.l.c.

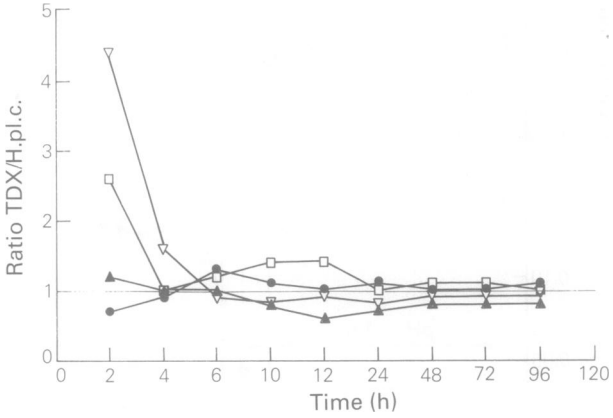

Figure 4 Average ratio of urine concentration measurement by h.p.l.c. and FPI *vs* time after drug administration. □ = capsules; ▲ = i.v.; ● = enteric-coated capsules; ▽ = tablets.

inter- and intra-individual variability and there was no statistical significance by overall analysis of variance.

Cardiovascular effects

The intravenous treatment had significantly greater effects on systolic blood pressure, reduced T wave amplitude and ST segment level, and reduced LVETI and QS_2I more, compared with the combined oral treatments (data not shown). There were no differences among the three oral treatments. In particular, the enteric-coated capsule did not have reduced effects on any of the measurements with the exception of the period to 120 min when the QS_2I was shortened more by the other oral treatments.

Discussion

In this study we have shown *in vitro* that digoxin undergoes extensive hydrolytic degradation at the pH conditions which prevail in the stomach, and that this occurs within a time compatible with the gastric residence of a tablet or capsule. This process would not be detected by the standard dissolution tests, since these require measurement of digoxin with a non-selective fluorometric method, with which the formulations used in this study would all have excellent dissolution profiles, with release of the full dose of digoxin within 10 min.

The breakdown products of this hydrolytic process are rapidly excreted in the urine and digitoxigenin has considerably less, if any, cardiac activity compared with digoxin (Böttcher *et al.*, 1973; Lullmann & Peters, 1971; Marcus *et al.*, 1975). All immunoassays show some cross-reactivity with these products. The pre-systemic breakdown of digoxin is therefore not reflected in the measured serum drug concentrations, which likely consist of a mixture of unchanged digoxin and breakdown products.

These metabolites are not exclusively formed by hydrolysis since evidence from radiotracer studies (Gault *et al.*, 1982; Magnusson *et al.*, 1982) indicates that hepatic metabolism of digoxin also occurs. An indication of this can be obtained in this study from the

urinary recovery of unchanged digoxin after intravenous administration. On average 68% of the total dose was recovered in a sampling period covering 2–3 half-lives, during which theoretically 75–87.5% should be recovered. This indicates that most, but not all, of the infused digoxin is excreted unchanged.

The recovery as a percentage of the administered dose after intravenous administration measured by FPI was lower, which is unexpected in view of the lack of selectivity of this assay. In a number of cases the urine samples at later times contained very low digoxin concentrations which could barely be measured by h.p.l.c. but not by FPI. This resulted in a lower recovery by FPI. Additionally, the varying cross-reactivity of metabolites that occur in different proportions during the collection period makes interpretation of the differences in recovery difficult.

It is common practice to evaluate the systemic availability of digoxin preparations on the basis of the serum drug concentration-time curve. Our data show that this can lead to erroneous results. Judging from the AUC measured by the non-selective FPI assay the systemic availability of the enteric-coated capsule would have been considered to be much reduced, while the capsule produced higher digoxin concentrations than the tablet, especially at earlier times, although this increased availability could not be demonstrated over the full 96 h sampling period by either method. The h.p.l.c. method was not sufficiently sensitive to allow measurements in serum, but the urinary recovery of unchanged digoxin from the three oral treatments was similar. The higher availability from the tablets and capsules compared with the enteric-coated capsules, measured by FPI in plasma, is therefore probably an artefact, caused by cross-reacting hydrolytic metabolites formed during absorption. The formation of these compounds was prevented by the enteric coating, which only allowed release of digoxin in the neutral environment distal to the stomach. Evidence for this also came from the overestimation of urine concentrations by FPI at early times, suggesting the presence of other immunoreactive substances, especially for the uncoated oral treatments. Although this effect failed to reach significance, we made a similar observation during an earlier study of the effects of gastric acidity on digoxin systemic availability (Cohen *et al.*, 1991).

The enteric-coated capsule was expected to increase the systemic availability of unchanged digoxin, but this did not happen. It is possible that this was due to the experimental formulation. In the first place, to ensure comparability, it was decided to coat the commercially available capsules and this required the administration of ten capsules for a dose of 1 mg. This may have resulted in release of drug at variable times from the different capsules, although this apparently did not happen after the uncoated capsules, which were otherwise similar. In addition, the lag phase of 1 h, and the late peak plasma drug concentrations at 4 h suggest that some drug release may have occurred in the colon, where absorption could have been low and where breakdown to dihydrodigoxin (Lindenbaum *et al.*, 1981) may have taken place. It is possible that further pharmaceutical development of this formulation could produce better systemic availability. In any case, it appears that an enteric-coated capsule will protect digoxin against breakdown in the stomach, and this may remove an important source of variability in the amount of digoxin available for absorption.

The liquid-filled digoxin capsules have been introduced as completely absorbed digoxin preparations (Doherty *et al.*, 1984; Johnson *et al.*, 1976), but our study indicates that this may be questionable.

Examination of the literature describing the increased systemic availability, shows that the increased plasma drug concentration appears to occur only in the first hours after administration (Bustrack *et al.*, 1984; Doherty *et al.*, 1984; Johnson *et al.*, 1976; Mallis *et al.*, 1975). This would be compatible with greater production of more rapidly cleared hydrolytic breakdown products from the capsule preparation, which may have been more susceptible to acid hydrolysis than the tablet. The pharmacokinetics of all of the hydrolytic breakdown products are not known but clearance of the monodigitoxoside (Kuhlmann *et al.*, 1974) and digoxigenin (Loo *et al.*, 1977) is faster than that of digoxin. Greater absorption of unchanged digoxin cannot be totally excluded, but in our study the increased peak plasma drug concentrations in the first 2 h seemed to be confirmed by the immunological assay. This resulted in greater (but not significantly so) digoxin excretion over 96 h. However, this was not confirmed when unchanged digoxin was assayed by h.p.l.c., which again suggests an artefactual result. Another advantage that has been suggested for the capsule formulation is reduced variability in plasma drug concentrations (Johnson *et al.*, 1986). This may be the result of more reproducible hydrolysis and could be interpreted as an artefact. The final evaluation of these points requires measurement of unchanged digoxin in plasma and developments toward such a method have been described recently (Embree & McErlane, 1989).

The shortening of the systolic time interval (Forester *et al.*, 1974; Weissler *et al.*, 1964) and the effects on the ST segment of the ECG and on blood pressure were as expected from the literature. Our measurements were sufficiently sensitive to demonstrate the greater effects of intravenous treatment in comparison with the oral treatments. The fact that there were no detectable differences between the oral treatments provides further indirect evidence that the differences in plasma drug concentrations between the treatments may have been caused by cardio-inactive metabolites. The actual amount of hydrolytic metabolites in urine is very low (1–2% by tracer methods). We actually do not know what appears in the urine. This is indirect evidence that whatever appears is inactive.

The implications of our findings could be of importance for the use of digoxin. Elderly patients, in whom achlorhydria is common, may absorb considerably more unchanged digoxin. This might not be disclosed by conventional therapeutic monitoring of plasma drug concentrations. Additionally, considerably greater variability of unchanged digoxin dosage may occur routinely, depending on gastric pH and the residence time of dosage forms in the stomach. Digoxin is metabolised to a greater extent than may be generally appreciated and it is possible that this compromises the value of therapeutic monitoring, for example in patients

with renal failure who are known to accumulate metabolites of digoxin (Gibson & Nelson, 1980).

Our data suggested that the presumed superior systemic availability of digoxin from liquid-filled capsules must be regarded with some scepticism, until this is proven by specific measurement of digoxin.

We gratefully acknowledge Mr G. de Maaker and Dr A. Moolenaar for assistance with the FPI digoxin determinations.

References

Bottcher, H., Lullmann, H. & Proppe, D. (1973). Comparison of digoxin with some digitoxin metabolites on cat heart lung preparation. *Eur. J. Pharmac.*, **22**, 109–111.

Bustrack, J. A., Katz, J. D., Hull, J. H., Foster, J. R., Hammond, J. E. & Christenson, R. H. (1984). Systemic availability of digoxin capsules and tablets: effect of co-administered fluid volume. *J. pharm. Sci.*, **73**, 1397–1400.

Cohen, A. F., Kroon, J. M., Schoemaker, H. C., Hoogkamer, J. F. W. & Vliet-Verbeek, A. (1991). Influence of gastric acidity on the systemic availability of digoxin. *Ann. Intern. Med.*, **115**, 540–545.

Doherty, J. E., Marcus, F. I. & Binnion, P. F. (1984). A multicenter evaluation of the absolute systemic availability of digoxin dosage forms. *Curr. Ther. Res.*, **35**, 301–306.

Embree, L. & McErlane, K. (1989). Development of a high-performance liquid chromatographic post-column fluorogenic assay for digoxin in serum. *J. Chromatogr.*, **496**, 321–334.

Forester, W., Lewis, R. P., Weissler, A. M. & Wilkie, T. A. (1974). The onset and magnitude of the contractile response to commonly used digitalis glycosides in normal subjects. *Circulation*, **49**, 517–521.

Gault, H., Kalra, J., Ahmed, M., Kepkay, D. & Barrowman, J. (1980). Influence of gastric pH on digoxin biotransformation. I. Intragastric hydrolysis. *Clin. Pharmac. Ther.*, **27**, 16–21.

Gault, H., Kalra, J., Ahmed, M., Kepkay, D., Longerich, L. & Barrowman, J. (1981). Influence of gastric pH on digoxin biotransformation. II. Extractable urinary metabolites. *Clin. Pharmac. Ther.*, **29**, 181–190.

Gault, H., Kalra, J., Longerich, L. & Dawe, M. (1982). Digoxigenin biotransformation. *Clin. Pharmac. Ther.*, **31**, 695–704.

Gibson, T. P. & Nelson, H. A.(1980). The question of cumulation of digoxin metabolites in renal failure. *Clin. Pharmac. Ther.*, **27**, 219–223.

Johnson, B. F., Bye, C., Jones, G. & Sabey, G. A. (1976). A completely absorbed oral preparation of digoxin. *Clin. Pharmac. Ther.*, **19**, 746–751.

Johnson, B. F., Lindenbaum, J., Budnitz, E. & Marwaha, R. (1986). Variability of steady-state digoxin kinetics during administration of tablets or capsules. *Clin. Pharmac. Ther.*, **39**, 306–312.

Kuhlmann, J., Abshagen, U. & Rietbrock, N. (1974). Pharmacokinetics and metabolism of digoxigenin-monodigitoxoside in man. *Eur. J. clin. Pharmac.*, **7**, 87–94.

Lindenbaum, J., Mellow, M. H., Blackstone, M. O. & Butler, V. P., Jr. (1971). Variation in biologic availability of digoxin from four preparations. *New Engl. J. Med.*, **285**, 1344–1347.

Lindenbaum, J., Rund, D. G., Butler, V. P., Jr., Tse Eng, D. & Saha, J. R. (1981). Inactivation of digoxin by the gut flora: reversal by antibiotic therapy. *New Engl. J. Med.*, **305**, 789–794.

Loo, J. C. K., McGilveray, I. J. & Jordan, N. (1977). Quantitation of digoxigenin in serum following oral administration of digoxin in humans. *Res. Comm. Chem. Path. Pharmac.*, **17**, 497–506.

Lullmann, H. & Peters, T. (1971). The cardioactivity of digitoxin metabolites. *Eur. J. Pharmac.*, **14**, 204–205.

Magnusson, J. O., Bergdahl, B., Bogentoft, C., Jonsson, U. E. & Tekenbergs, L. (1982). Excretion of digoxin and its metabolites in urine after a single oral dose in healthy subjects. *Biopharm. Drug Dispos.*, **3**, 211–218.

Mallis, G. J., Schmidt, D. H. & Lindenbaum, J. (1975). Superior systemic availability of digoxin solution in capsules. *Clin. Pharmac. Ther.*, **18**, 761–768.

Marcus, F. I., Ryan, J. N. & Stafford, M. G. (1975). The reactivity of derivatives of digoxin and digitoxin as measured by the Na-K-ATPase displacement assay and by radioimmunoassay. *J. lab. clin. Med.*, **85**, 610–620.

Shepard, T. A., Hui, J., Chandrasekaran, A., Sams, R. A., Reuning, R., Robertson, L. W., Caldwell, J. H. & Donnerberg, R. L. (1986). Digoxin metabolites in urine and faeces. A fluorescence derivatization-high performance liquid chromatography technique. *J. Chromatogr.*, **380**, 89–98.

Sonobe, T., Hasumi, S., Yoshino, T., Kobayashi, Y., Kawata, H. & Nagai, T. (1980). Digoxin degradation in acidic dissolution medium. *J. pharm. Sci.*, **69**, 410–413.

Warrington, S. J., Turner, P. & Skrumsager, B. K. (1989). Cardiovascular (ECG and systolic time intervals) and anticholinergic effects of repeated doses of femoxitine—a comparison with amitryptiline and placebo in healthy men. *Br. J. clin. Pharmac.*, **27**, 343–351.

Weissler, A. M., Gamel, W. G., Grode, H. E., Cohen, S. & Schoenfeld, C. (1964). The effect of digitalis on ventricular ejection in normal human subjects. *Circulation*, **19**, 721–729.

(Received 9 April 1992, accepted 8 July 1992)